

EHV-1 vaccination is performed to ameliorate clinical respiratory disease, late term abortion and myelopathy. Inactivated virus vaccines and few modified-live virus vaccines are available. A product in Europe claiming to aid in abortion prevention has a high antigenic load and manufacturer-specific adjuvants (Equip<sup>®</sup>EHV-1,4; Zoetis). While few horses develop local swelling or fever after IM administration, this is likely unacceptable for horse owners, and could lead to decreased compliance with vaccination. However, a post-vaccination systemic inflammatory response (SIR) could also indicate a more robust specific immunity. Therefore, we hypothesised that horses with detectable SIR will have higher virus-neutralising (VN) antibody titres than horses without detectable SIR. We also tested whether differences exist between vaccinated mules and horses. Adult mules ( $n=11$ ) and horses ( $n=11$ ), previously vaccinated with an inactivated EHV-1/EHV-4 vaccine (Equip<sup>®</sup>EHV-1,4) at 6-month intervals on at least two occasions were used. Booster vaccination was performed on day 0 (d0) of the study. Before and daily post vaccination for 7 days, rectal temperature, nasal discharge and mandibular lymph node (MLN) size were recorded. Blood was collected prior to vaccination (d0), on days 3, 5, 7, 14, 21, and additionally when fever was detected. Serum amyloid A (SAA) was measured on d0 – d7 as a marker of SIR. VN titres were measured on d0, 7, 14 and 21. Normality was assessed with the Shapiro-Wilk-test. Mann-Whitney-test was applied to compare horses to mules. A Spearman-correlation-coefficient ( $R^2$ ) between the maximum increase in VN titres and the highest SAA value was calculated. Significance was pre-set at  $p<0.05$ . Pre-vaccination clinical exams were all normal. A single episode of fever ( $>38.0^\circ\text{C}$ ) occurred in 4/11 (36%) horses and 2/11 (18%) mules (total fever days per subject = 1). All subjects showed seromucous nasal discharge for 7 days. MLN were unremarkable throughout the study. All subjects had increased post-vaccination SAA concentration ( $>1.7\text{mg/L}$ ) on at least one occasion. VN results (median [interquartile range]) were significantly different ( $P=0.0058$ ) between horses (1:256 [1:64; 1:256]) and mules (1:512 [1:256; 1:512]) on day 7. A statistically significant ( $P<0.05$ ) change in antibody titres compared to baseline (d0) was observed in mules on d7. No significant difference in SAA between horses and mules was recorded. A significant increase in SAA was observed on d3 ( $P<0.001$ ) and d5 ( $P<0.01$ ) compared to d0 in both groups. No correlation was observed between peak SAA and the increase in VN titres in horses ( $R^2=0.09$ , CI: -0.58-0.65;  $P=0.80$ ) or mules ( $R^2=0.06$ , CI: -0.55-0.67;  $P=0.86$ ). In conclusion, vaccination induced SIR characterised by significantly increased SAA, but the intensity of SIR was not associated with a higher VN antibody response. The immune response differed between groups, with higher antibody production in mules. Overall, the absence of a significant post-vaccination rise in VN titres suggests that the adequacy of current vaccination programs should be further investigated.

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### Susceptibility of equid herpesvirus 3 field isolates to antiviral compounds

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#### Efficacy of antivirals expressed as percentage of inhibition

	ACV	GCV	HPMPC
Reduction of EHV3 production after 48h	>88%	>87%	>58%
Reduction of plaque size	84-100%	84-92%	76-85%
Reduction of plaque number	76-96%	58-93%	13-47%

Equine Coital Exanthema (ECE) is an infectious, venereally transmitted mucocutaneous disease of mares and stallions caused by equid herpesvirus 3 (EHV3). Prevention in stallions is based on segregating affected mares from reproduction. However, reactivated virus from latently infected mares is re-excreted subclinically, and therefore the prevention approach previously described does not eliminate the risk of contagion. Outbreaks of ECE have a negative impact on horse reproductive practices; there is thus an urgent need of a specific method of treatment and prevention. In previous studies, we demonstrated the efficacy of antiviral compounds, such as acyclovir (ACV), ganciclovir (GCV) and cidofovir (HPMPC), against EHV3 reference strain. The aim of the present work was to evaluate these compounds against different field isolates of EHV3 *in vitro*. Six EHV3 isolates were obtained from affected mares in Argentina from 2007 and 2008. They were selected according to phenotypic and genetic differences determined prior to this study in order to form a group of viruses representative of the variety of the circulating EHV3. Monolayers of EDerm cells in 12- wells tissue culture plates were infected with 30 plaque forming units/well of each field strain. After two h. of incubation, overlay medium, with and without carboxymethyl cellulose (CMC) 0.75%, supplemented with ACV 5 µg/ml, GCV 0.05 µg/ml and HPMPC 2 µg/ml was added. The two sets of plaques were incubated at 37°C in a CO<sub>2</sub> incubator for 72 h. The same experiment with each viral isolate but without the addition of antiviral compound was performed as control. To quantify EHV3 production, aliquots of the supernatant (without CMC) were taken at 48 and 72 h after infection, and DNA was extracted and analyzed by a quantitative real time PCR (qPCR) targeting the gG gene. To determine plaque size and plaque number, the cells with CMC 0.75% were fixed and stained after 72 h with 0.1% formalin-buffered crystal violet solution. Results shown in the following table are the mean value of the three independent experiments.

In conclusion, the susceptibility of EHV3 field isolates to ACV, GCV and HPMPC was similar among them, and the results are in concordance with the ones obtained with the reference EHV3 strain. The antiviral effect of these compounds was similar for plaque size, but for plaque number and EHV3 production, HPMPC was the least effective, being ACV and GCV similar regarding their effectiveness against EHV3. However, as GCV was the most efficient compound, it will be considered to be evaluated topically on experimentally infected mares.

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### Survey of equine herpesviruses-1, -2, -4 and -5 in 407 horses with and without respiratory disease

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Equine herpesviruses (EHVs) are common respiratory pathogens in horses and other equids, which are responsible for serious health, welfare and financial consequences worldwide. The